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ELECTRICAL ACTIVITY IN RESPONSE TO LIGHT OF THE OCELLUS OF THE HYDROMEDUSAN, *SARSIA TUBULOSA*

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Abstract

The electroretinogram (ERG) recorded from the ocellus of *Sarsia tubulosa* has a characteristic positive potential change at the onset of illumination, followed by a slower biphasic pulse and a positive deflection at the cessation of illumination succeeded by high-frequency pulses. The amplitude of initial pulse is graded with respect to changes in intensity and wavelength of the light stimulus. The maximum spectral sensitivity lies around 540 nm. Responses to light with opposite polarity were recorded from the optic ganglion which surrounds the ocellus. Differences in response patterns inside and outside the receptive field of the ocellus were mapped. Morphological structures which could give rise to component responses of the ERG and its neuronal pathway are discussed.

Introduction

Ocelli associated with tentacular structures are common in coelenterates. The number of such ocelli varies from four [*e.g. Sarsia tubulosa* (Sars, 1835)] to sometimes several hundred [*e.g. Spirocodon saltatrix* (Tilesius, 1818)]. The structural complexity of ocelli varies widely from simple epithelium not elaborated into a cup, but with photoreceptor cells (Singla, 1974), to a highly organized eyecup with a lens or a lens-like structure (Weber, 1981a, b). Several types of structures between these two extremes have been reviewed by Yoshida (1973) and Singla (1974).

The eyecup (retina) in *Sarsia* (Fig. 1; Singla and Weber, in preparation) is composed of pigment and photoreceptor cells in almost a 1:2 ratio. The photoreceptors are bipolar and grouped together. Bundles of 2 to 10 long (up to 50 μm in length) distal receptor processes project their apical cilia into the lumen of the ocellar cavity (about 90 μm deep and 50–60 μm wide). The plasma membrane covering the cilium forms lateral microvilli. The cell bodies of photoreceptors lie behind the pigmented cup, 20 to 60 μm from the ocellar cavity. The proximal region of photoreceptors extends as an axon. Synapses occur between neighboring cell bodies and axons of the receptor cells at the optic ganglion which surrounds the ocellus (Mackie, 1971). Second order neurons, as described in other hydromedusae (Toh, *et al.*, 1979; Yamamoto and Yoshida, 1980; Singla and Weber, 1982) have not been observed. Instead, the proximal axons of the bipolar receptor cells group together to form a pair of optic nerves (Singla and Weber, in preparation) which travel around the tentacle base on either side and enter the tentacular ganglion (Mackie, 1971), a swelling of the outer nerve ring.

While most invertebrates have photoreceptors of the rhabdomeric type, all the photoreceptors of jellyfish that have been examined so far are of the ciliary type (Eakin and Westfall, 1962; Eakin, 1963). According to Eakin's theory (1963, 1968,

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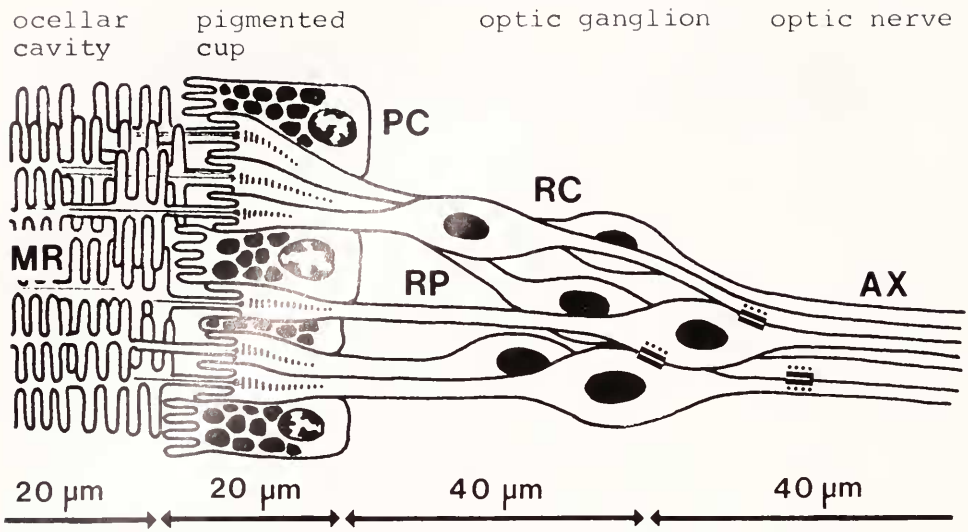

FIGURE 1. Summary diagram of receptor cell arrangement in the ocellus of *Sarsia tubulosa*. AX, axon; MR, microvilli region; PC, pigment cell; RC, receptor cell; RP, receptor cell process.

1979) of the two lines of evolution of photoreceptors, the photoreceptor cells of Cnidaria belong to the same evolutionary line as those of vertebrates. While the rhabdomeric photoreceptors respond with a depolarization, cilia-derived photoreceptors are hyperpolarizing in general. The question then arises whether the physiology of *Sarsia's* receptors is also like that of vertebrate receptors?

In this paper I describe the electroretinogram of a jellyfish, *Sarsia tubulosa* (Hydromedusae), and map the different patterns of photo-induced responses at the optic ganglion which surrounds the receptive field of the ocellus.

MATERIALS AND METHODS

Specimens of *Sarsia tubulosa* (M. Sars) were collected from the water around Victoria, B. C., Canada and from the dock at the Friday Harbor Laboratories of the University of Washington. They were either maintained in cool, running sea water and exposed to daylight or transferred to a holding tank in a dark room at 8°C. The water in the tank was continuously replenished with fresh, filtered seawater.

For electrical recording, whole or partially dissected animals were pinned down on a silicone elastomer plastic (#184; Sylgard, Dow Corning Corp.) layer in the recording dish. Experiments were done in seawater at 8 to 10°C. Recordings were made from ocelli which had been dark-adapted for at least 15 min. (This time for full dark adaptation was determined experimentally). The extracellular recordings were made with plastic suction electrodes with external tip diameters ranging from 20 to 25 μm and with glass microelectrodes (Ultra Tip, Federick Haer & Co., #30-30-0) with external tips of 5 to 15 μm in diameter. The shaft of the latter was connected to the amplifier input lead by an intermediate piece of polyethylene tube. Both types of suction electrodes were filled with seawater, and both allowed vigorous movements of the preparations without electrical artifacts. The recording electrodes were placed with a micromanipulator in the center of the ocellus (unless otherwise noted) while the indifferent electrode was placed into the bathing medium. By

adding isotonic magnesium chloride in suitable portions the amount of muscular activity was reduced.

Electrical responses were fed into a Grass 79C polygraph via capacity-coupled (AC) amplifiers with a half-amplitude fall time constant of 600 msec. The amplified signal was passed through a 5 Hz low pass filter and displayed concurrently on a storage oscilloscope (Tektronix 5111).

For photo-stimulation, light from a tungsten-halogen lamp (150W/120V) was passed through a camera shutter and brought by a single optical fibre to an evenly illuminated spot (approximately 100 μm in diameter), covering the entire retina. The intensity of the light stimulus was controlled by calibrated neutral density filters (Zeiss) placed in the beam beyond the shutter. An optical fibre placed beyond the neutral density filters led to a photocell which monitored the light stimulus. For light measurements a QSL-100 Laboratory Quantum Scalar Irradiance Meter was used. The unattenuated light intensity, measured at the level of the ocellus, was equivalent to 0.7×10^{13} quanta $\cdot$ sec$^{-1} \cdot$ cm^{-2}. Intensities (I) of light stimuli are expressed in log units relative to this value (*e.g.* for a full intensity stimulus, log I = 0.0; for a flash attenuated by a factor of 100, log I = -2.0).

Spectral sensitivities of ocelli were determined by measuring the response amplitude (% of maximum) to 60 msec light stimuli at different wavelengths from 350 to 700 nm with constant intensity. The output of monochromatic light (Balzers color filters) at different wavelengths was measured with a quantum meter, and sensitivity measurements were corrected for the differences in energy with calibrated neutral density filters.

Control responses to a stimulus of a given intensity and wavelength were measured at various times during the experiments to check that sensitivity was not changing with time.

Results

Response pattern to brief and long light stimuli recorded from the center (30 μm in diameter) of the ocellus

The ocellus of *Sarsia tubulosa* responded to a brief light flash (log I = 0.0; 10 to 50 msec in duration) with a single transient positive potential change. This on-response had a latency of 35 to 45 msec and a duration of 200 to 300 msec. The amplitude ranged from 40 to 80 μV (Fig. 2A). The on-response often was followed by a small undershoot of variable amplitude, rarely followed by a slow positive deflection. The undershoot became more pronounced in recordings with closely apposed plastic suction electrodes than with the thinner glass electrodes. It also gradually increased when the electrodes were shifted out of the center of the ocellar cup.

The response pattern to long light stimuli (in the range of 1 sec and longer; log I = 0.0) consisted of an initial transiently positive potential change, identical to the on-response to brief light flashes. It was followed by a second slower biphasic pulse of variable amplitude and with a long recovery phase (Fig. 2B). Whereas the on-response remained stable when light flashes were repeated at intervals of 45 sec or more, the biphasic pulse became smaller due to light adaptation. The off-response was a slow positive deflection approximately 1 sec in duration with a latency of 200 msec. This was often followed by series of small high frequency pulses (with durations ranging from 150 to 300 msec) for up to 60 sec (Fig. 2B).

Glass suction electrodes lightly attached to the center of the ocellar cavity

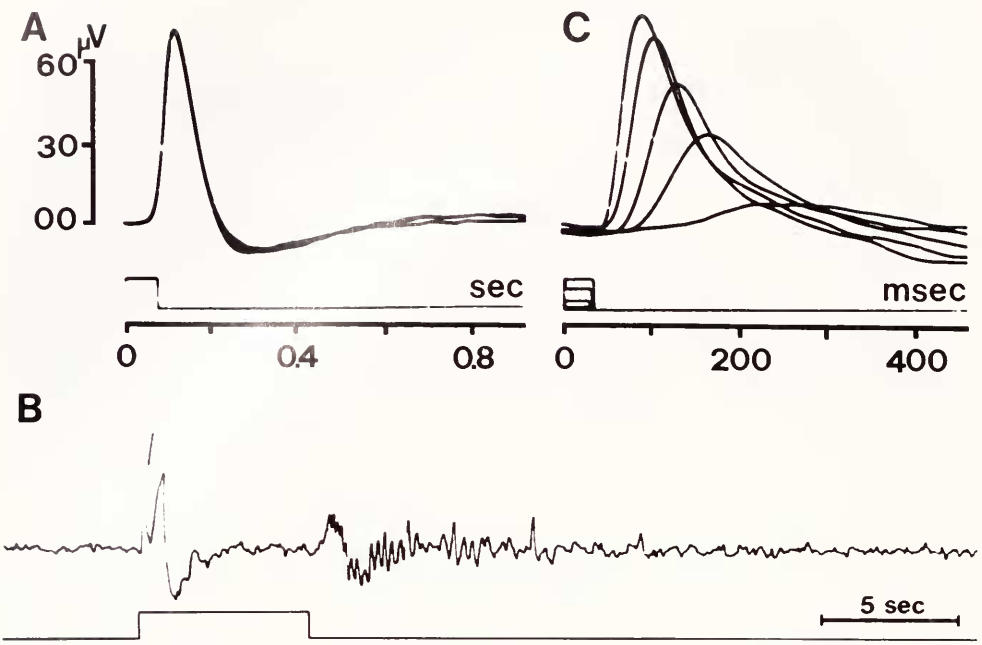

FIGURE 2. Responses to brief and long light stimuli recorded from the center of the ocellus. Lower trace shows signal from photocell monitoring the light stimulus. (A): superimposed oscilloscope traces of on-response to three 60 msec light stimuli (log I = 0.0) at intervals of 30 sec. (B): response pattern to a long light stimulus. The first positive potential change is followed by a second biphasic pulse with a long recovery phase. The off-response is a slow positive deflection followed by a series of high frequency pulses. (C): graded responses of the ocellus to 30 msec light stimuli of increasing intensity. Superimposed oscilloscope traces of responses to five light stimuli. Intensity of successive flashes increases from log I = −3.0 (smallest response); −2.0; −1.5; −0.75 and 0.0 (largest response). Interval between stimuli 60 sec.

recorded the on-response but not the biphasic pulse (Fig. 6B), and the high frequency pulses following the off-response were either very small or missing.

No changes in response pattern to brief and to long light stimuli were recorded from animals kept either in the dark or in the light for at least 12 hours after 90 sec in the dark.

Sensitivity to light and time course of responses

The amplitude of the positive on-response was graded with respect to the intensity of the light. Figure 2C shows superimposed responses to five 30 msec light stimuli of increasing intensity. The relative intensity ranged from log I = −3.0 (smallest response) to log I = 0.0 (largest response). The interval between successive light flashes was 60 sec. The response amplitude reached a maximum at a relative stimulus intensity of about log I = 0.0. Further increases in stimulus intensity usually elicited no further increases in response magnitude. The latency of onset and time to peak of the response decreased with increasing light intensity. The shortest latency recorded for the on-response was 35 msec at log I = 0.0.

Spectral sensitivity

Figure 3 shows the spectral sensitivity curve of the on-response amplitudes expressed as a percentage of the maximum response for ten ocelli against the

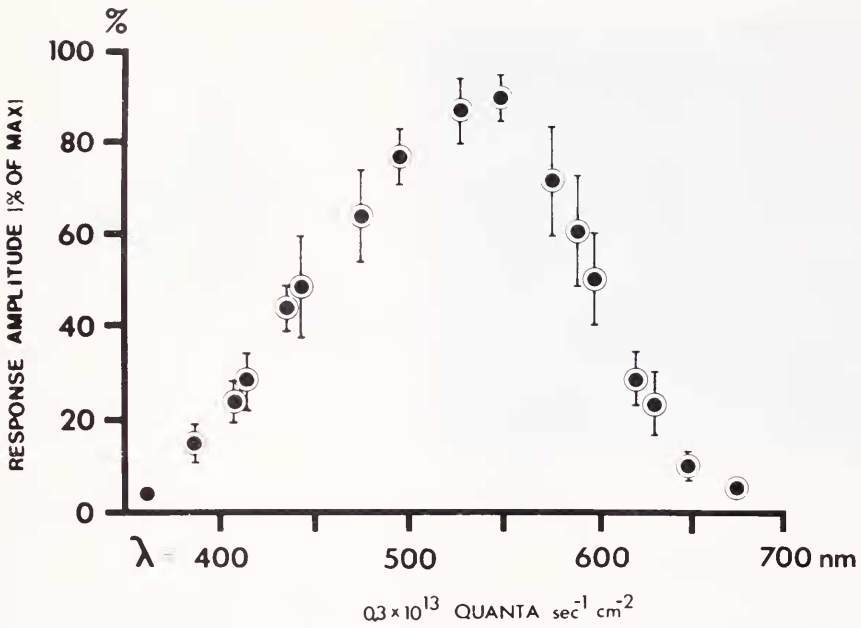

FIGURE 3. Spectral sensitivity of the ocellus of *Sarsia tubulosa*. Mean value (⊙) of response amplitudes expressed as a percentage of the maximum response for ten ocelli against the different wavelengths (nm). Error bars indicate standard deviation.

different wavelengths (nm). Changes of the maximum response amplitudes during each series were less than 5%. The light intensity measured at the level of the ocellus was 0.3×10^{13} quanta $\cdot$ sec^{-1} $\cdot$ cm^{-2}. The spectral sensitivity of the ocelli ranged from λ = 363 nm to 675 nm with its maximum around 540 nm.

Changes in response patterns at different sites of the ocellus

Whereas the above described response patterns to brief and long light stimuli were restricted to the center of the ocellar cavity (Fig. 4A), recordings to brief light stimuli (log I = 0.0) from sites adjacent to the center showed smaller on-response amplitudes. In fact, the amplitude of the positive on-response gradually decreased with increasing distance of the recording electrode from the center. At the periphery of the pigmented cup the on-response could not be recorded. Beyond the edge of the pigmented ring which surrounds the ocellar cavity a negative on-response was recorded (Figs. 4B, C). This negative response increased with distance, reaching a maximum at 60 to 70 μm from the center of the ocellus. A small negative response could be recorded as far as 100 to 120 μm from the center (Fig. 4D).

To analyze whether the two on-responses of opposite polarity were due to the same receptive structure, responses to light stimuli were recorded simultaneously from different sites of the ocellus. Figure 5A shows superimposed oscilloscope traces to three 30 msec light stimuli (log I = 0.0) at intervals of 60 sec. One glass suction electrode was lightly attached to the center of the ocellus while the other was attached laterally to the optic ganglion (approximately 90 μm apart). In all cases, the positive and negative deflections induced by light stimuli were correlated with each other (Fig. 5A). If the electrodes were attached to the optic ganglion at opposite sites (approximately 50 and 70 μm from the center) the latency of onset and time to peak of the negative events was proportional to the distance between

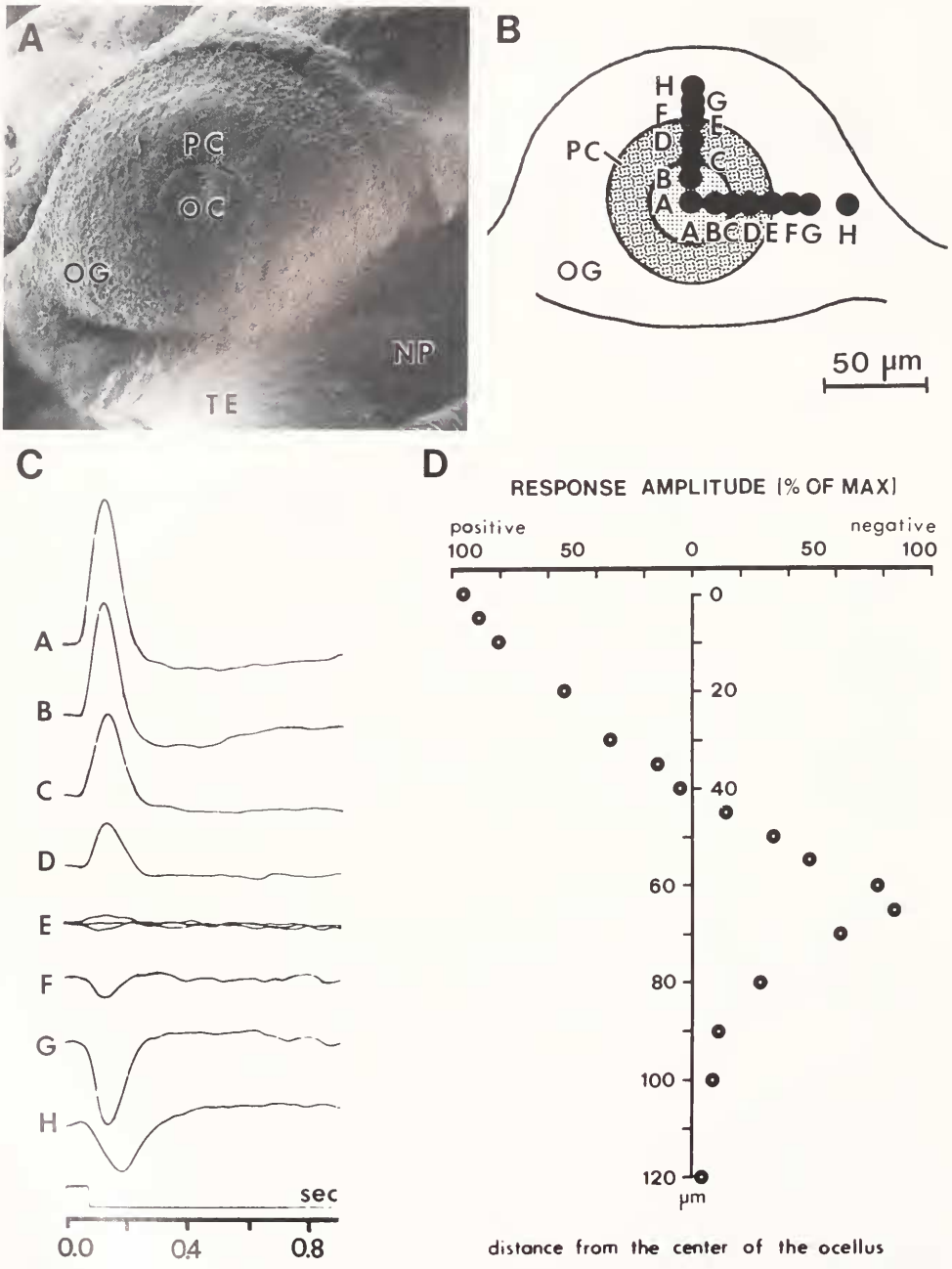

FIGURE 4. Structure of ocellus and summary diagram of changes in responses to brief light stimuli (log I = 0.0) at different sites of the ocellus. (A): scanning electron micrograph of the ocellus of *Sarsia tubulosa*. NP, nematocyst pad; OC, ocellar cavity; OG, optic ganglion; PC, pigmented cup; TE, tentacle. Same magnification as in B. (B): diagram of the ocellus. Each black dot represents the approximate site of a recording electrode. Their distribution pattern is circular. (C): sequence of records, A, B, . . . H, correspond with the sites labelled in B. (D): plot of peak response expressed as a percentage of the maximum response against the horizontal distance from the center of the ocellus. Each point is the average of three to eight measurements.

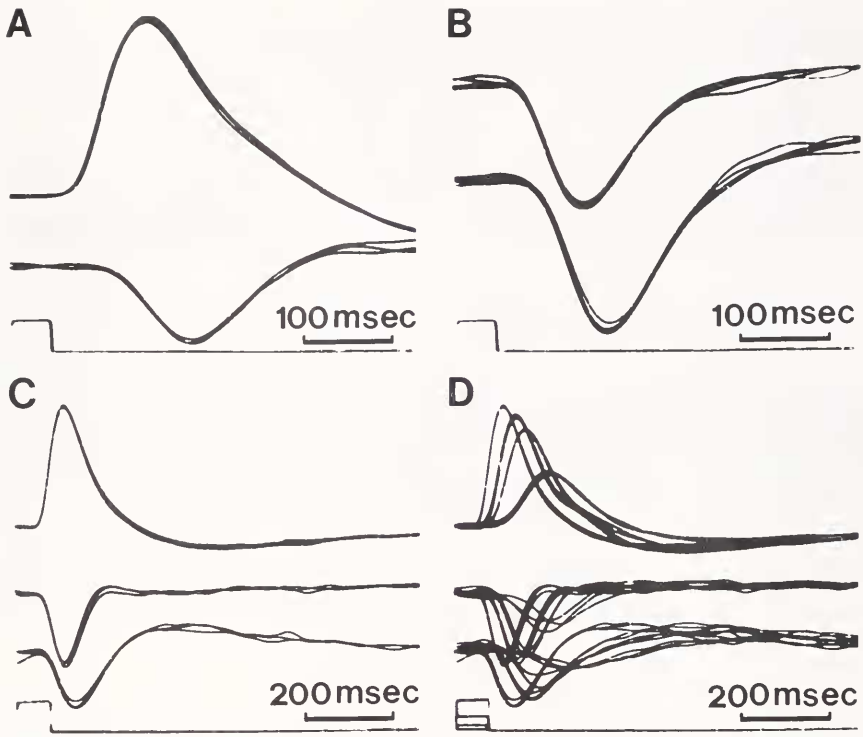

FIGURE 5. (A): superimposed oscilloscope traces of responses to three 30 msec light stimuli at intervals of 60 sec. Records are from the center of the ocellus (positive response) and from the optic ganglion (negative response). (B): superimposed oscilloscope traces of responses to five 30 msec light stimuli. Records of two electrodes attached to the optic ganglion at opposite sites, approximately 50 μm (upper trace) and 70 μm (lower trace) from the center of the ocellus. (C): superimposed traces of responses to three 70 msec light stimuli. One electrode is placed in the center of the ocellus (upper trace) and two electrodes are attached to the optic ganglion, approximately 60 μm (middle trace) and 80 μm (lower trace) from the center. (D): superimposed traces of responses to light stimuli with decreasing intensity. Intensity of successive flashes decreases from log I = 0.0 (largest response); −1.0; −1.5; −3.0 (smallest response). Interval between stimuli 60 sec. Same setting of electrodes as in C. Bottom trace in A, B, C and D shows signal from photocell monitoring the light stimulus. Time measured from beginning of light flash.

the electrodes and the center of the ocellus (Fig. 5B). Recordings with three electrodes, attached to the center and to two different sites of the optic ganglion (approximately 60 and 80 μm from the center; Fig. 5C, D), also demonstrated striking correspondence in changes of response amplitudes to light stimuli of full intensity (Fig. 5C) and to stimuli with decreasing intensity (Fig. 5D).

To compare the response patterns from different sites to brief and long light stimuli, recordings were made simultaneously from the center of the ocellus and from the optic ganglion. Whereas the on-response to brief light stimuli recorded from the ocellus sometimes was followed by a slow positive deflection of variable amplitude, its counterpart recorded from the optic ganglion never included a comparable second event following the slight overshoot of the initial negative potential change (Fig. 6A). The response pattern of the optic ganglion to long light stimuli, however, resembled a reflected image of the response pattern recorded from the center of the ocellar cavity (Fig. 6B), except that the off-response recorded from

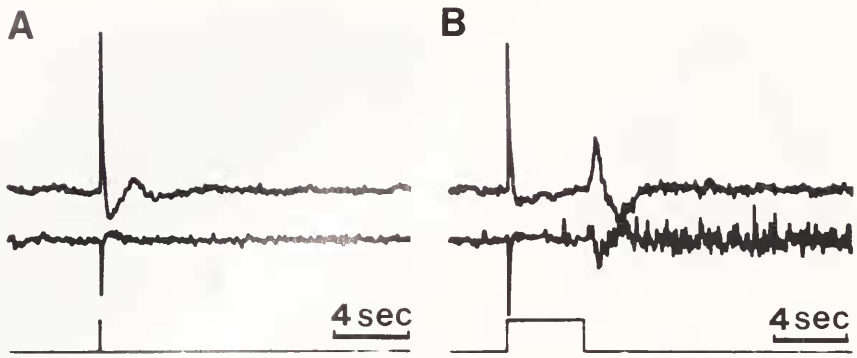

FIGURE 6. Comparison between different response patterns to brief and long light stimuli. (A): recordings from the center of the ocellus (upper trace), and from the optic ganglion (lower trace) to a brief light stimulus (log I = 0.0). (B): same recording sites. Response pattern to a long light stimulus. The high frequency pulses following cessation of illumination recorded from the optic ganglion (lower trace) are conspicuous by their absence in recordings from the center of the ocellus (upper trace). Bottom trace in A and B shows signal from photocell monitoring the light stimulus.

the optic ganglion was succeeded by a series of high frequency pulses which were absent in the center of the ocellus under identical recording conditions (glass suction electrodes).

DISCUSSION

The electroretinogram (ERG) recorded from the center of the ocellus of *Sarsia tubulosa* has a characteristic positive potential change at the onset of illumination, followed by a slower biphasic pulse with a long recovery phase and a positive off-response succeeded by a series of high frequency pulses. This response pattern is strikingly similar to the ERG from the ocellus of the jellyfish *Polyorchis penicillatus* (Weber, unpublished). The most conspicuous features of the on-response are: (1) the relation between its latency and the intensity of the light stimulus, and (2) the graded amplitude with respect to the light intensity and to photic stimulation with different wavelength at constant intensity. The ocellus is most sensitive to blue-green and green light. The spectral sensitivity curve ranges from 363 nm to 675 nm with a maximum around 540 nm.

The use of glass suction microelectrodes with small tip diameters allows splitting of the ERG into component responses and mapping of changes in the ERG at different sites inside and outside the pigmented cup of the ocellus. The results suggest that the positive on-response is mainly due to photo-induced receptor potentials of photoreceptors, and that the presumed receptor potential originates at the center of the ocellar cavity and propagates in all directions. The amplitude of the response decreases with increasing distance from the center. Beyond the edge of the pigmented cup, it increases again but with opposite polarity, then decreases again (Fig. 4). It is still to be determined with correlative work at the intracellular level whether a reverse of the sign of photocurrent along photoreceptors, similar to that found in retinae of vertebrates, cephalopods, and invertebrates (Hagins, *et al.*, 1962; Penn and Hagins, 1969; Shaw, 1972), is responsible for the response patterns of opposite polarity. The physiological observations, however, suggest that *Sarsia*'s photoreceptors hyperpolarize to light as vertebrate receptors do, and that they conduct decrementally.

Histological observations on the ocellus of *Sarsia* show that the cell bodies of photoreceptors lie at various levels behind the ocellar cup, up to 60 μm from the ocellar cavity. The proximal axons of the bipolar receptor cells group together to form a pair of optic nerves (Singla and Weber, in preparation) which encircle the tentacle base and enter the tentacular ganglion (Mackie, 1971). Synapses are common at the optic ganglion between receptor cell bodies, between axons and receptor cell bodies, and among axons (Fig. 1). In this context it is of special interest that in most experiments in which fine glass suction electrodes were used, the series of high frequency pulses following the off-response can only be recorded from the optic ganglion (Fig. 6B). These high frequency pulses probably result from activity in the nervous plexus of the optic nerves following cessation of illumination. It is uncertain, however, whether these post-stimulus fast potentials are identical to pre-swim pulses recorded from *Sarsia* (Passano, 1976).

The ERG can be recorded out to the edge of the optic ganglion. No responses to light stimuli are recorded from the nematocyst pad and the exumbrella surrounding the optic ganglion. Recordings from the tentacular ganglion, which is situated below the tentacle base, do not show any activity directly correlated with the ERG. It is still to be determined whether "swimming" in *Sarsia* is triggered by the photo-induced responses via direct neuronal pathways (optic nerves) between receptor and effector organ, as appears to be the case in other hydromedusae (Yoshida and Ohtsu, 1973; Anderson and Mackie, 1977).

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